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Fish Acute Toxicity Syndromes Application to the Development of Mechanism-Specific QSARS (Quantitative Structure Activity Relationships)

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by

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FISH ACUTE TOXICITY SYNDROMES: APPLICATION TO THE DEVELOPMENT OF MECHANISM-SPECIFIC QSARS.

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ABSTRACT

In the field of aquatic toxicology a number of groups have proposed and are using predictive models, based on quantitative structure activity relationships (QSARs), as rapid screening tools to identify potentially hazardous chemicals. Several QSARs are now available that predict the acute toxicity of narcotic-industrial chemicals. Predictions for compounds acting through more specific modes of action, which are generally more toxic, require alternative models. Implementation of mechanism-specific quantitative models will require the development of qualitative models that assign the proper mode of action, and respective QSAR, to a given chemical. Current efforts in developing the components of this model are focused on characterizing a toxic mode of action database from which the structural requirements for specific mechanisms can be elucidated. Approaches to defining modes of action include application of joint toxicity theory for chemical mixtures and the assessment of fish acute toxicity syndromes (FATS). FATS are distinct sets of *in vivo* rainbow trout (*Salmo gairdneri*) toxic responses that correspond to specific modes of action. By measuring a number of respiratory-cardiovascular variables, response sets associated with nonpolar and polar narcotics, oxidative phosphorylation uncouplers, respiratory membrane irritants, respiratory inhibitors, acetylcholinesterase inhibitors, and central nervous system seizure agents have been described.

INTRODUCTION

Under the Toxic Substances Control Act the U.S. Environmental Protection Agency (EPA) is charged with the responsibility of assessing the hazards of chemicals to human health and the environment. Through the premanufacture notice (PMN) process EPA must review and assess the potential hazard of a new chemical within 90 d, generally with little more than the compound's structure available (DiCarlo et al., 1985). With over 50,000 compounds currently in the inventory of U.S. industrial chemicals (McCutcheon, 1980) and with more than 1,200 chemicals per year submitted to EPA under the PMN review process (DiCarlo et al., 1985), reliable and efficient methods are needed to identify potentially hazardous chemicals for extensive evaluation. In the field of aquatic toxicology, a number of research groups have proposed the use of predictive models based on quantitative structure activity relationships (QSARs), as rapid screening tools to identify potentially hazardous chemicals (Konemann, 1981a,b; Veith et al., 1983).

Several QSARs are now available that predict the acute toxicity of narcotic-industrial chemicals to aquatic species (Konemann, 1981a; Veith et al., 1983). These narcosis models predict only baseline toxicity. Predictions for compounds acting through more specific modes of action, which are generally more toxic, require alternative models (Hermens, 1986). Implementation of mechanism-specific quantitative structure toxicity models will require the development of qualitative structure activity models that assign the proper mode of action, and respective QSAR, to a given chemical. Current efforts in developing the components of this model are focused on characterizing a toxic mode of action database from which the structural

requirements for specific mechanisms can be elucidated. Approaches to defining common modes of action include application of joint toxicity theory for chemical mixtures (Broderius and Kahl, 1985; Veith and Broderius, 1987) and the assessment of fish acute toxicity syndromes (FATS).

As described in a recent review (McKim et al., 1987c), FATS are distinct sets of in vivo rainbow trout (Salmo gairdneri) toxic responses that correspond to specific modes of action. By measuring a number of respiratory-cardiovascular variables, FATS associated with nonpolar and polar narcotics, oxidative phosphorylation uncouplers, respiratory membrane irritants, acetylcholinesterase (AChE) inhibitors, and the pyrethroid insecticide fenvalerate have been described (McKim et al., 1987a,b; Bradbury et al., 1987; Bradbury et al., 1988). Preliminary investigations with respiratory inhibitors and central nervous system seizure agents have also been completed (unpublished data).

APPROACH

The general approach to assemble and employ the FATS database is presented in Figure 1.

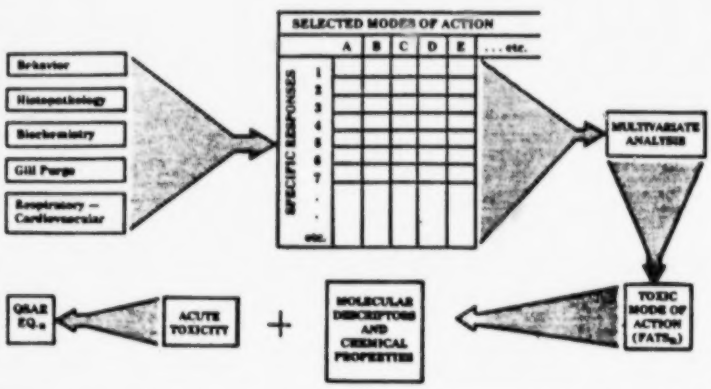

Figure 1. Flow chart relating FATS to the generation of QSAR equations for predicting acute toxicity of organic chemicals to fish.

In vivo rainbow trout toxic responses are measured during aqueous exposure to chemicals with known modes of action. The large number of responses monitored, and their interdependence, results in a complex matrix of quantitative and qualitative data. By using discriminant function analysis (DFA) (Tatsuoka, 1971), these complex data sets can be simplified and the best response variables for predicting a specific FATS can be determined. Once the relationship between chemical structure and mode of action can be identified, the appropriate molecular descriptors, chemical properties, and corresponding toxicity databases can be analyzed to assemble the associated structure-toxicity models.

The experimental approach to assess the physiological response of rainbow trout to intoxication by selected chemicals has been described previously (McKim et al., 1987a,b; Bradbury et al., 1987). Briefly, spinally-transected trout (0.6 to 1.0 kg) are exposed to lethal concentrations of the selected toxicant in specially designed respirometer-metabolism chambers and a series of physiological variables are monitored until death. Those variables typically used in the statistical analyses are listed in Table 1.

Table 1. Physiological variables monitored in rainbow trout to define FATS.

Variable		Units
Ventilation Volume	(V_G)	ml/min
Total Oxygen Consumption	($\dot{V}O_2$)	mg/kg/h
Gill Oxygen Uptake Efficiency	(U_E)	%
Ventilation Frequency	(f_V)	no./min
Cough Frequency	(f_C)	no./min
Heart Frequency	(f_H)	no./min
Total Blood Oxygen (arterial)	(TaO_2)	g/100 mL
Total Blood Carbon Dioxide (arterial)	($TaCO_2$)	mmol/L
Blood pH (arterial)	(pHa)	pH units
Hematocrit	(Hct)	%
Hemoglobin	(Hb)	g/100 mL

Preceding toxicant exposure, measurements for all variables are made on each fish (N = 4 per chemical and a separate control group of N = 4) to provide predose values. The mean percentage change of physiological parameters from their predose mean is determined for the 25 to 75% survival time interval for each fish. Measurements within this interval are used to minimize inclusion of responses due to initial stress and death itself. These data are then used in DFA to identify which of the physiological variables best separate responses of fish exposed to chemicals representing different FATS.

CURRENT STATUS

Five FATS have now been definitively described. These FATS are associated with nonpolar, or Type I narcotics (Veith and Broderius, 1987), polar narcotics (weakly acidic or basic aniline and phenol derivatives with log Ps generally less than 2.9), Type II narcotics (Veith and Broderius, 1987), oxidative phosphorylation uncouplers, AChE inhibitors, and respiratory membrane irritants.

The overall response of trout to the Type I narcotic chemicals, MS-222 and 1-octanol (McKim et al., 1987a), was a dramatic slowing of all respiratory-cardiovascular functions (Fig. 2). While ventilation volume and oxygen consumption decreased, oxygen uptake efficiency increased as water flow over the gills slowed and the blood-to-water perfusion ratio increased. Ventilation frequency declined initially and then increased slowly until death. This increase in ventilation frequency was simply hyperventilation as little or no water was being pumped across the gills. A rapid drop in heart frequency (reflex bradycardia) was likely related to the increase in vagal tone caused by hypoxia. As respiration declined, water flow over the gills was reduced and total arterial oxygen and arterial pH decreased. In response to hypoxia, hematocrit increased substantially, while hemoglobin increased only slightly. Increased hematocrit (brought on by red blood cell swelling), during anesthesia, is a well-documented response to hypoxia. In separate experiments it was established that these primary intoxication responses were reversible, which fulfills an important criterion for defining the observed responses as a narcotic effect (Kaufman, 1977). Generally, nonpolar narcosis is consistent with depressant anesthesia as described by Winters (1976).

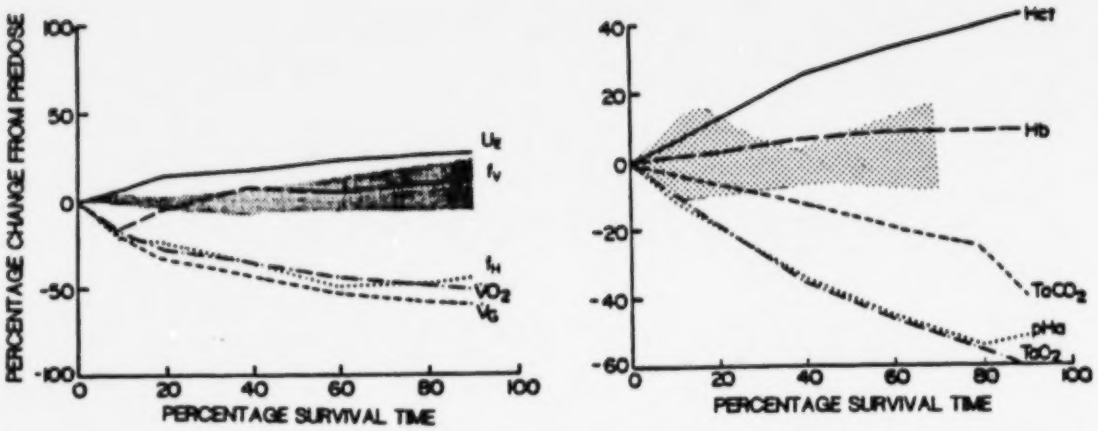

Figure 2. Physiological responses of rainbow trout representative of intoxication by nonpolar (Type I) narcotics. Stippled area represents the maximum range of responses from control fish. Each line is the mean measurement of four fish. See Table 1 for abbreviations.

The most striking characteristic associated with intoxication by Type II narcotics (phenol, 2,4-dimethylphenol, aniline, 2-chloroaniline, and 4-chloroaniline) (Bradbury et al., 1988) was the development of tremors and clonic seizures which were initiated with coughs. Between 1.5 and 10% survival time the fish exhibited tremors that originated in the head and then moved posterially to the tail. Between 10 and 30% survival time the tremors progressed to periods of clonic seizures, which culminated in episodes where the tail was raised out of the water. As the seizures became more intense the trout became unresponsive to outside stimuli. The intensity and severity of the seizures increased through approximately 60% survival time after which they gradually subsided and the fish became mostly inactive. Interestingly, the seizures involved the entire fish even though the trout had been spinally transected. The response of these polar narcotics suggests that they were affecting the central nervous system, including the spinal cord posterior to the transection, or perhaps the peripheral nervous system. In mammals, the primary site of phenol stimulation in the central nervous system is thought to be the spinal cord. Tremors in mammals are seemingly due to reflex stimulation, while clonic convulsions result from increased sensitivity of the motor mechanism of anterior horn cells (Deichmann and Keplinger, 1981). Changes in rainbow trout physiological parameters (Fig. 3) were consistent with the increased muscular activity associated with seizures, as measured by cough frequency. These changes were generally reflected by an increased oxygen demand to support the heightened activity as demonstrated by initial rises in oxygen consumption. Ventilation volume and oxygen consumption eventually declined, probably as a result of the increased frequency and severity of the seizures, which made respiration erratic and inefficient. Continued declines in ventilation volume and oxygen consumption reflected an overall depression of respiratory activity with increasing exposure consistent with a general narcotic effect. The major changes in blood-chemistry parameters (i.e., decreasing arterial oxygen, carbon dioxide, and pH and increasing hematocrit) suggested a rapid shift toward anaerobic metabolism, which would be consistent with the initially heightened muscular activity during seizures and the subsequent depression. Using phenol as a model agent, the reversibility of the visible signs of intoxication and the

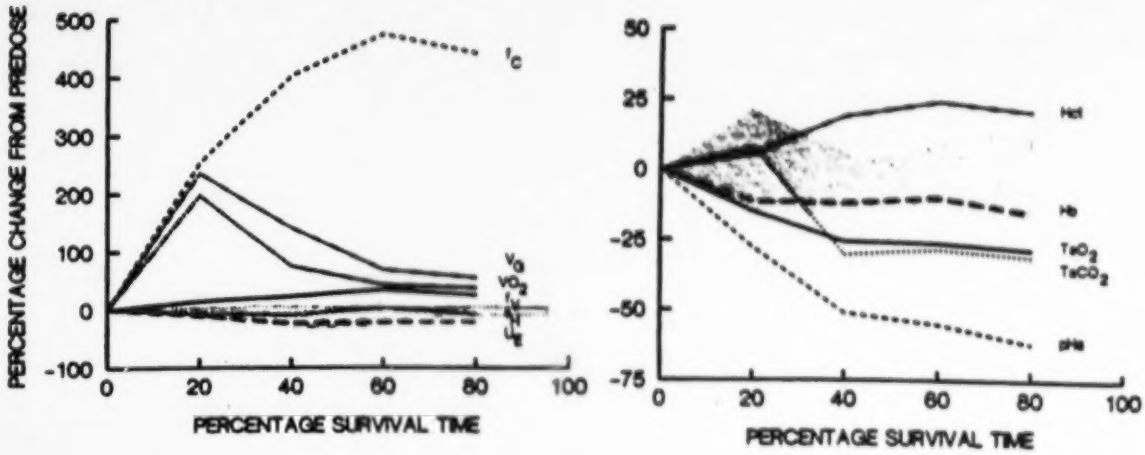

Figure 3. Physiological response of rainbow trout representative of intoxication by polar (Type II) narcotics. See Fig. 2 legend for an explanation of symbols, etc.

primary respiratory-cardiovascular responses were also established. Trout could be brought to the point of respiratory collapse and then revived by artificial irrigation of the gills. The toxic responses associated with Type II narcotics are consistent with the description of cataleptic anesthesia put forth by Winters (1976).

The most impressive diagnostic response representative of the respiratory uncoupler syndrome chemicals (pentachlorophenol and 2,4-dinitrophenol) was a rapid and continuous increase in ventilation volume and oxygen consumption over the entire survival period (Fig. 4) (McKim et al., 1987a). These responses corresponded to a continuously rising metabolic rate, which was not impacted to any degree by increased activity since the exposed animals were immobile. Interestingly, the increase in ventilation volume and oxygen consumption did not include a change in either oxygen uptake efficiency or ventilation frequency.

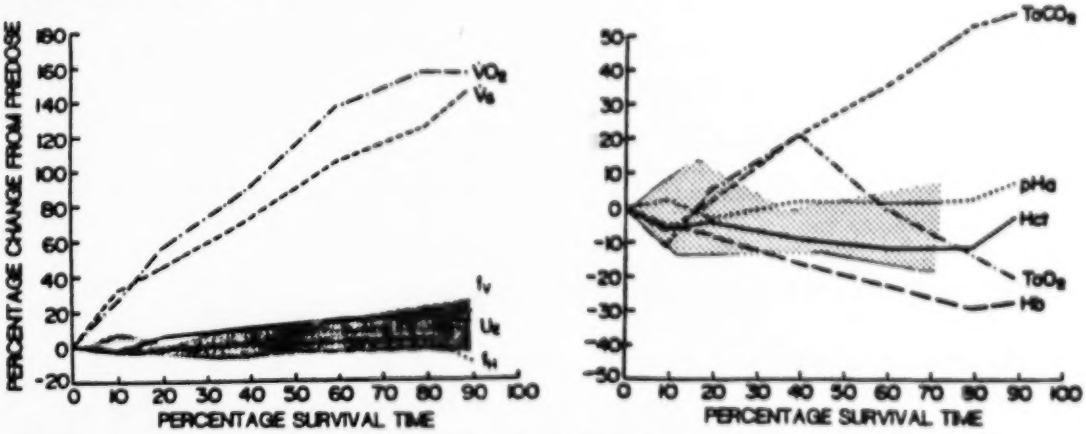

Figure 4. Physiological responses of rainbow trout representative of intoxication by oxidative phosphorylation uncouplers. See Fig. 2 legend for an explanation of symbols, etc.

The trout were therefore increasing the water flow across their gills by increasing the ventilatory stroke volume, while at the same time maintaining a constant percentage removal of oxygen at the lamellar surface. The elevated oxygen consumption was reflected by an initial increase in total arterial oxygen content that lasted for almost half of the survival period and then fell slowly, presumably as the tissues used more oxygen in an effort to generate ATP.

The most striking respiratory-cardiovascular responses (Fig. 5) noted for the AChE inhibitor syndrome chemicals, malathion and carbaryl, were immediate decreases in oxygen uptake efficiency and heart frequency (McKim et al., 1987b).

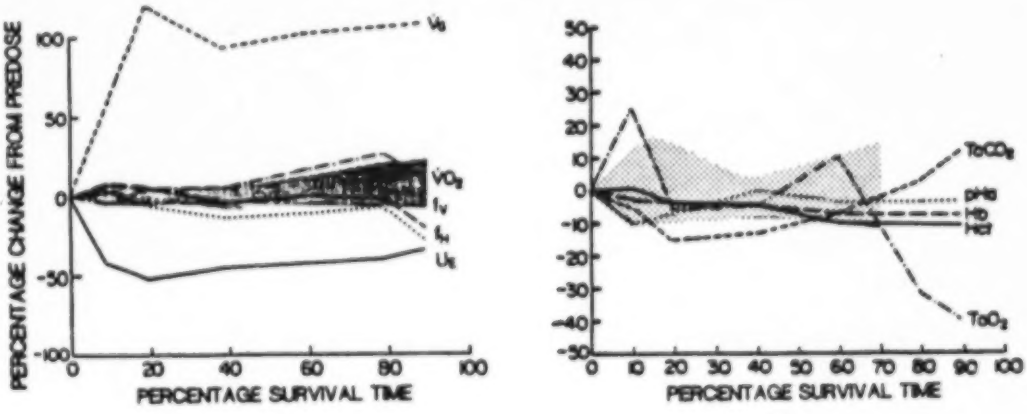

Figure 5. Physiological responses of rainbow trout representative of intoxication by AChE inhibitors. See Fig. 2 legend for an explanation of symbols, etc.

Ventilation volume seemingly increased to compensate for the lower oxygen uptake efficiency, but not enough to increase total oxygen consumption. The reduction in uptake efficiency with a compensatory rise in ventilation volume can perhaps be explained by the action of acetylcholine on neural-muscular junctions in the gills, resulting in a reduced blood flow through the secondary lamellae. Reduction in heart frequency in malathion and carbaryl intoxicated fish was likely due to excessive inhibition of the heart by the vagus nerve (mediated by cholinergic synapses).

The overall respiratory-cardiovascular responses (Fig. 6) representative of the respiratory irritant syndrome chemicals, acrolein and benzaldehyde, indicated a direct toxic effect on gill structure. Accompanying an initial rapid increase in cough frequency was an initially moderate-to-low increase in ventilation volume and total oxygen consumption. However, at 50 to 60% survival time a consistent drop in ventilation volume and total oxygen consumption was observed. Ventilation frequency, oxygen uptake efficiency, and heart frequency showed a steady downward trend over the entire survival period. Total arterial oxygen and carbon dioxide, and arterial pH decreased midway through the the survival time, while hematocrit increased steadily and hemoglobin remained within the control range. These respiratory-cardiovascular responses provide evidence supportive of a general hypoxic effect caused by direct gill damage.

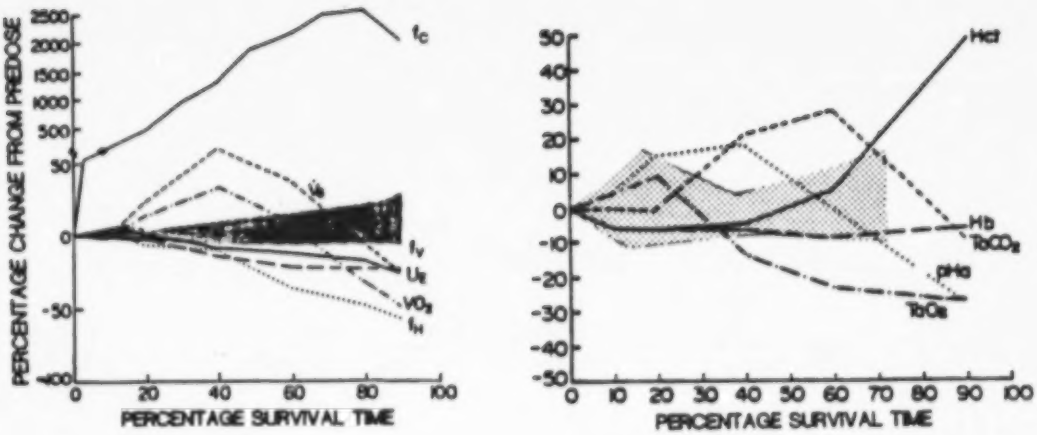

Figure 6. Physiological responses of rainbow trout representative of intoxication by respiratory membrane irritants. See Fig. 2 for an explanation of symbols, etc.

Based on the toxic responses described above, DFA was used to assess whether the fish exposed to the 13 chemicals could be separated into five distinct FATS (Bradbury et al., 1988): Type I narcosis syndrome, Type II narcosis syndrome, a respiratory uncoupler syndrome, an AChE inhibitor syndrome, and a respiratory membrane irritant syndrome. The use of DFA with the 52 trout resulted in 100% correct classification of five FATS (Fig. 7). Of the 11 responses measured and used in the DFA, cough frequency, arterial pH, ventilation volume, oxygen consumption, and heart frequency were the best discriminators to use to define the five FATS and they discriminated 49 of the 52 fish (94%). Three additional variables, ventilation frequency, arterial oxygen, and hemoglobin were required to achieve 100% discrimination. Cough frequency was the best discriminating variable, separating respiratory irritant and Type II narcotic exposed fish from the other three FATS groups because only the former chemicals elicited cough/seizure responses. Arterial pH was the next best discriminating variable, separating Type II narcotic from irritant exposed fish. These two variables alone resulted in the discrimination of 90% of the Type II narcotic exposed fish and 65% of the 52 fish. The third and fourth best discriminating variables were ventilation volume and oxygen consumption which further separated the Type I narcotic, Type II narcotic, uncoupler, and AChE inhibitor exposed fish. The ability to discriminate between the five FATS is not necessarily dependent on the availability of one important variable such as cough frequency. Excluding cough frequency in the DFA, 94% (49 of 52) of the fish were discriminated into the respective FATS and arterial pH, ventilation volume, oxygen uptake efficiency, ventilation frequency, arterial oxygen, and hemoglobin were the variables included in the model.

In addition to the toxicants and FATS just described we have also developed preliminary data sets to evaluate syndromes associated with respiratory inhibitors and central nervous system seizure agents. A critical aspect of these studies is to determine at what level the approach can discern between different molecular mechanisms. Investigations with seizure agents include response sets associated with pyrethroid (fenvalerate and cypermethrin) and cyclodiene (endosulfan and endrin) insecticides, and strychnine, whose molecular modes of action are associated with effects

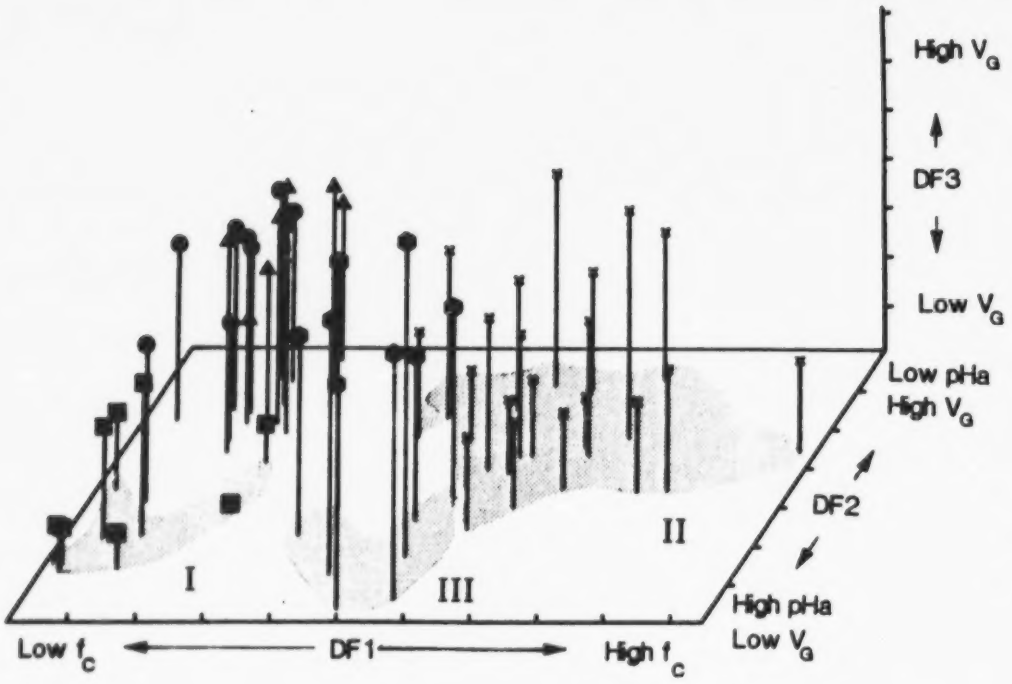

Figure 7. Three-dimensional plot of the first three discriminant functions that separate five FATS - nonpolar narcosis (I), polar narcosis (II), respiratory irritant (III), AChE inhibitor, and respiratory uncoupler syndromes. The latter two syndromes are correctly resolved as well (see text); however, because of scaling for the plot the separation is not readily discerned visually.

on sodium channels, the GABA-receptor-ionophore complex, and glycine receptors, respectively. Preliminary studies with fenvalerate (Bradbury et al., 1987) and endosulfan and strychnine (unpublished data) indicate that these response sets are unique to the original five FATS. Additional testing with cypermethrin and endrin will permit an evaluation as to whether these agents can be resolved on the basis of molecular mechanism (i.e., into three distinct FATS). Similar investigations with respiratory inhibitors have also been initiated. Testing with rotenone established a response set unique to the original FATS (unpublished data). Examination of additional blockers will further assess the specificity of the approach.

FUTURE DIRECTIONS

Development of a database large enough to provide the basis from which to determine those properties and characteristics that are crucial in predicting mode of action obviously requires approaches supplemental to that of the FATS assessment. Currently, we are employing joint toxicity theory for chemical mixtures as a way of screening larger numbers of chemicals to identify and assign modes of action. We are also reviewing the U.S. EPA, ERL-Duluth

fathead minnow acute toxicity database of over 400 chemicals, and based on expert assessment, assigning chemicals to modes of action as appropriate. When this exercise is complete we plan to use this test data set and assess the use of graph-theoretic indices (Basak et al., 1988; Basak et al., 1987; Niemi et al., 1985) to determine the nature of structural similarity with chemicals from specific mode of action groups. It is hoped that this exercise will provide an initial step in developing an expert system to predict mode of action from chemical structure.

REFERENCES

Basak, S.C., V.R. Magnuson, G.J. Niemi and R.R. Regal. 1988. Determining structural similarity of chemicals using graph-theoretic indices. Discrete Appl. Math. In press.

Basak, S.C., V.R. Magnuson, G.J. Niemi, R.R. Regal and G.D. Veith. 1987. Topological indices: Their nature, mutual relatedness, and applications. Math. Model. 8:300-305.

Bradbury, S.P., J.M. McKim and J.R. Coats. 1987. Physiological response of rainbow trout (Salmo gairdneri) to acute fenvalerate intoxication. Pestic. Biochem. Physiol. 27:275-288.

Bradbury, S.P., T.R. Henry, G.J. Niemi, R.W. Carlson and V.M. Snarski. 1988. Use of respiratory-cardiovascular responses of rainbow trout (Salmo gairdneri) in identifying acute toxicity syndromes in fish: Part 3. Polar narcotics. Submitted to Environ. Toxicol. Chem.

Broderius, S.J. and M. Kahl. 1985. Acute toxicity of organic chemical mixtures to the fathead minnow. Aquat. Toxicol. 6:307-322.

Deichmann, W.B. and M.L. Keplinger. 1981. Phenols and phenolic compounds. In: G.D. Clayton and F.E. Clayton, eds., Patty's Industrial Hygiene and Toxicology, 3rd ed., John Wiley and Sons, New York, NY, pp. 2567-2627.

DiCarlo, F.J., P. Bickart and C.M. Auer. 1985. Role of the structure-activity team (SAT) in the premanufacture notification (PMN) process. In: M. Tichy, ed., QSAR in Toxicology and Xenobiochemistry, Elsevier, Amsterdam, Holland, pp. 433-449.

Hermens, J.L.M. 1986. Quantitative structure-activity relationships in aquatic toxicology. Pestic. Sci. 17:287-296.

Kaufman, R.D. 1977. Biophysical mechanisms of anesthetic action: Historical perspective and review of current theories. Anesthesiology 46:49-62.

Konemann, H. 1981a. Quantitative structure-activity relationships in fish toxicity studies. Part 1: Relationship for 50 industrial pollutants. Toxicology 19:209-221.

Konemann, H. 1981b. Fish toxicity tests with mixtures of more than two chemicals: A proposal for a quantitative approach and experimental results. Toxicology 19:229-238.

McCutcheon, R.S. 1980. Toxicology and the law. In: J. Doull, C.D. Klaassen and M.D. Amdur, eds., Casarett and Doull's Toxicology: The Basic Science of Poisons, 2nd ed., Macmillan, New York, NY, pp. 727-735.

McKim, J.M., P.K. Schmieder, R.W. Carlson, E.P. Hunt and G.J. Niemi. 1987a. Use of respiratory-cardiovascular responses of rainbow trout (Salmo gairdneri) in identifying acute toxicity syndromes in fish: Part 1. Pentachlorophenol, 2,4-dinitrophenol, tricaine methanesulfonate and 1-octanol. Environ. Toxicol. Chem. 6:295-312.

McKim, J.M., P.K. Schmieder, G.J. Niemi, R.W. Carlson and T.R. Henry. 1987b. Use of respiratory-cardiovascular responses of rainbow trout (Salmo gairdneri) in identifying acute toxicity syndromes in fish: Part 2. Malathion, carbaryl, acrolein and benzaldehyde. Environ. Toxicol. Chem. 6:313-328.

McKim, J.M., S.P. Bradbury and G.J. Niemi. 1987c. Fish acute toxicity syndromes and their use in the QSAR approach for hazard assessment. Environ. Health Perspect. 71:171-186.

Niemi, G.J., R.R. Regal and G.D. Veith. 1985. Applications of molecular connectivity indexes and multivariate analysis in environmental chemistry. In: J.J. Breen and P.E. Robinson, eds., Environmental Applications of Chemometrics, ACS Symposium Series 292, American Chemical Society, Washington, D.C., pp. 148-159.

Tatsuoka, M.M. 1971. Multivariate Analysis. John Wiley and Sons, New York, NY.

Veith, G.D. and S.J. Broderius. 1987. Structure-toxicity relationships for industrial chemicals causing Type(II) narcosis syndrome. In: K.L.E. Kaiser, ed., QSAR in Environmental Toxicology-II, D. Reidel Publishing Co., Dordrecht, Holland, pp. 385-391.

Veith, G.D., D.J. Call and L.T. Brooke. 1983. Structure-toxicity relationships for the fathead minnow: Narcotic industrial chemicals. Can. J. Fish. Aquat. Sci. 40:743-748.

Winters, W.D. 1976. Effects of drugs on the electrical activity of the brain: Anesthetics. Annu. Rev. Pharmacol. Toxicol. 16:413-426.